



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of

Mamoru OHASHI et al

Serial No : 09/529,715

Filed : April 19, 2000

For : FAST-DISSOLVING PHARMACEUTICAL COMPOSITION

Group Art Unit:1616

Examiner : Sharmila Gollamudi

DECLARATION (C)

Honorable Commissioner of  
Patents and Trademarks  
Washington, DC 20231

Sir:

I, Mamoru OHASHI, a citizen of Japan residing at 10-23-501, Ogaki 6-chome, Ritto-shi, Shiga-ken, Japan, declare as follows.

1. I was graduated from Kyoto Pharmaceutical University in March 1988, and completed the master's course at the same university in March 1990.

Since April 1990 up till the present, I have been an employee of Dainippon Pharmaceutical Co., Ltd. and had been engaged in researches and developments of drug product formulation, particularly in solid dosage form in Pharmaceutical Research Laboratory of said company.

2. I am one of the co-inventors for the invention described in U.S. Serial No. 09/529,715 and am familiar with the subject matter thereof.

3. I have read the cited Negoro et al., U.S. Patent 5,258,382, Muller et al., U.S. Patent 5,858,410, Arbuthnot et al., U.S. Patent 6,458,811 and Schneider et al., U.S. Patent 5,356,636 and am familiar with the subject matter thereof.

4. Under my supervision, the following experiment has been done for the purpose of comparison of the dissolving properties between a tablet containing nanoparticles of AS-3201 and a tablet containing microparticles of AS-3201.

Said AS-3201 means (R)-2-(4-bromo-2-fluorobenzyl)-1,2,3,4-tetrahydro-pyrrolo[1,2-a]pyrazine-4-spiro-3'-pyrrolidine-1,2',3,5'-tetraone.

**Experiment 1:** Try to prepare nanoparticles by dry pulverization method

(1) Micronizing by dry pulverization method (by Jet Mill)

AS-3201 crystals prepared in a similar manner as disclosed in Example 1 of Negoro et al., U.S. Patent 5,258,382 were micronized using Single Truck Jet Mill (manufactured by SEISHIN ENTERPRISE Co., Ltd.) with grinding air pressure of 7 kgf/cm<sup>2</sup> to give powders (Lot No. 96004).

The micronization with Jet Mill is carried out, in principle, by making coarse particles violently crushed against each other with high-speed air jet.

(2) Measurement of mean particle size by dry dispersion method

The mean particle size of the powders prepared in the above Experiment 1 (1) was measured by dry dispersion method three times. The results are shown in Table 1.

**Table 1:** Mean particle size of particles micronized by Jet Mill

	Number of Measuring			Mean
	1	2	3	
Particle Size (μm)	1.36	1.37	1.35	1.36

**Measuring method of particle size by dry dispersion method:**

The mean particle size was measured using a laser diffraction particle size distribution analyzer [HELOS (trademark), manufactured by SYMPATEC GmbH, Germany] with a RODOS dry-air dispersing module at 0.5 bars in dispersion air pressure, and calculated from cumulative particle distribution on volume basis.

(3) Sieving of microparticles

It was tried to obtain nanoparticles of AS-3201 by subjecting the microparticles of AS-3201 obtained in the above Experiment 1 (1) (means particle size: 1.36 μm, hereinafter occasionally expressed merely as "1.36 μm" unless otherwise specified) using a sieve having minimum opening size and thereby separating off microparticles contained in the products.

In more detail, the AS-3201 microparticles were sieved using 400 mesh sieve (sieve opening size 37  $\mu\text{m}$ ) and a ro-tap-type sieving shaker (Type ES-65, Iida Seisakusyo Co.,Ltd., Japan) for 60 minutes. The amount passed through the 400 Mesh screen was less than 0.1%. Thus, the microparticles could not sufficiently pass through the sieve due to clogging.

Since the microparticles did not pass through the sieve having such a large opening of about 27 times ( $= 37/1.36$ ) larger than the mean particle size of the AS-3201 microparticles, it would be impossible to get nanometer fraction with a sieve having smaller opening.

The clogging of sieve would be due to the electrical charge of the microparticles. That is, the microparticles have larger electrical charge with smaller of the particle size, which results in secondary flocculation of the particles and thereby the flocculated particles could not pass through the sieve. Thus, it would highly possibly be difficult to prepare the desired nanoparticles by dry pulverization method.

#### Experiment 2: Try to prepare nanoparticles by wet pulverization method

In view of failure to prepare the nanoparticles by dry pulverization method in Experiment 1 (2), it was tried to prepare them by wet pulverization method.

According to the wet pulverization method, it has been considered that the particles would hardly occur secondary flocculation because of elimination of the electrical charge due to the presence of water. Then, it was tried to micronize the particles with a machine similar to "Micron LAB 40" disclosed in Example 1 of Muller et al. U.S. Patent 5,858,410, i.e. with DeBEE 2000™ (BEE international Ltd.).

The micronization with DeBEE is carried out, in principle, by giving high-pressure to a suspension containing microparticles, wherein a high speed jet stream of water is created and then the coarse particles are micronized by shearing force and/or cavitation produced by the high speed jet stream of water.

The microparticles obtained in Experiment 1 (1) (1.36  $\mu\text{m}$ , 10 g) was added to water (100 ml) and the mixture was stirred in order to prepare a suspension containing microparticles. However, since the microparticles of AS-3201 have less wettability and dispersibility and hence could not homogeneously dispersed in water. Accordingly, the desired dispersion could not be obtained and hence the particles of AS-3201 could not be micronized by

DeBEE.

**Experiment 3: Preparation of nanoparticles by wet pulverization method and measurement of particle size**

**(1) Preparation of nanoparticles by wet pulverization method:**

In order to improve the wettability and dispersibility of the microparticles in the light of the results in the above Experiment 2, hydroxypropylcellulose (abbreviated as "HPC") was added. As a result, it was found that the microparticles of AS-3201 could be homogeneously dispersed in water.

Then, a mixture of the microparticles obtained in Experiment 1 (1) (1.36  $\mu\text{m}$ , 200 g), HPC (10 g) and water (1990 g) was stirred to give a suspension wherein the microparticles of AS-3201 were homogeneously dispersed (cf. attached Fig. 1, PHOTO B-1). Then the suspension was pulverized in the DeBEE homogenizer under operating pressure of 35,000 psi (2,413 bar), twenty (20) cycles. The state of the resultant suspension is shown in the attached Fig. 1, PHOTO C-1. The attached photographs are all at 150-fold magnification.

**(2) Measurement of particle size of the nanoparticles obtained in the above wet pulverization**

**(i) Preliminary experiment**

The particle size of the particles contained in the suspension obtained in the above Experiment 3 (1) was preliminarily measured as follows.

Since it was impossible to measure the sample containing water by dry dispersion method as disclosed in Experiment 1 (2), the particle size was measured by wet dispersion method.

It is known that the measurement of particle size by wet dispersion method gives different mean size in some degree from that measured by dry dispersion method. Accordingly, it was measured by both methods, i.e. dry dispersion method and wet dispersion method and the data thus obtained were compared. That is, with respect to the microparticles obtained in Experiment 1(1), the mean particle size was measured by dry dispersion method, and the same particles were suspension in water, and then the mean particle size of the suspension was measured by wet dispersion method. This procedure was repeated three times. The results are shown in Table 2.

**Measuring method of particle size by wet dispersion method:**

The particle size was measured using a laser diffraction particle size distribution analyzer (HELOS) with a SUCELL wet dispersing module. Sample was diluted and dispersed into 0.5% HPC solution corresponding to 25 µg/mL in the concentration of AS-3201 drug substance. The mean particle size was calculated from cumulative particle distribution on volume basis.

**Table 2:** Mean particle size of microparticles measured by dry dispersion method and wet dispersion method (µm)

Method for measurement	Number of Measuring			Mean
	1	2	3	
Dry dispersion method, RODOS module	1.36	1.37	1.35	1.36
Wet dispersion method, SUCELL module	2.05	2.09	2.04	2.06

As is seen from the results in Table 2, even in the same sample, the value of the mean particle size obtained by wet dispersion method was about 1.5 times larger than the data measured by dry dispersion method.

**(ii) Measurement of mean particle size of nanoparticles in suspension**

The mean particle size of nanoparticles in a suspension obtained in the above Experiment 3(1) was measured by wet dispersion method. The value obtained was corrected along with the knowledge found in the above (i). The results are shown in Table 3.

**Table 3:** Measured and corrected mean particle size of nanoparticles in suspension (nm)

Frequency of pulverization	0-Cycle	1-Cycle	2-Cycle	3-Cycle	5-Cycle	10-Cycle	20-Cycle
Measured value	2,060	1,170	1,120	1,110	1,070	1,020	950
Corrected value	1,360	770	740	730	710	670	630

"0(zero)-Cycle" means microparticles before pulverization (mean particle size: 1360 nm = 1.36 µm, see attached Fig. 1, PHOTO B-1). When the

microparticles were pulverized once (1-Cycle), they became particles having a mean particle size of 770nm, and when they were pulverized 20 times (20-Cycle), they became particles having a mean particle size of 630nm (see attached Fig. 1, PHOTO C-1). As is seen from said photograph, it is clear that the particles became smaller.

**Experiment 4:** Try to prepare powdery nanoparticles and measurement of particle size

**(1) Vacuum drying**

The nanoparticles in the suspension (800 g) obtained in Experiment 3(1) were gathered by using a centrifugal separator at 31,800 gravity in centrifugal force for 10 minutes. After decanted off a supernatant liquid, residual mass was dried using a vacuum drying oven at 60°C, -0.1 MPa for 16 hours. The dried particles were strongly agglomerate as a white cracker. The agglomerate was crushed in a mortar. The resultant particles were sieved through 60 mesh sieve (sieve opening size 250  $\mu\text{m}$ ), and then the mean particle size was measured by dry dispersion method. As the result, the mean particle size was 102  $\mu\text{m}$ .

**(2) Freeze dry (lyophilizing)**

(i) The suspension (565 g) containing nanoparticles (630 nm) obtained in Experiment 3 (1) was lyophilized by a conventional method (i.e. freeze-dried at -45°C and dried at 10Pa for about 24 hours) to give white powder of 50.2 g (yield 93.1 %). The particle size of said powder was measured by dry dispersion method. As the result, the mean particle size was 41.6  $\mu\text{m}$ . The view of the powder is shown in the attached Fig. 1, PHOTO C-2.

(ii) As a control, the suspension obtained in Experiment 3(1) before pulverization with DeBEE homogenizer (0-Cycle, 1.36  $\mu\text{m}$ ) was freeze-dried in the same manner as described in the above (i). The mean particle size of the resultant particles was measured by dry dispersion method. As the result, the mean particle size was 8.2  $\mu\text{m}$ . The view of the powder is shown in the attached Fig. 1, PHOTO B-2.

The suspension containing microparticles (1.36  $\mu\text{m}$ ) (PHOTO B-1) was re-powdered by lyophilization to give a powder (the view of the product is shown in the attached Fig. 1, PHOTO B-2) which had a mean particle size of 8.2  $\mu\text{m}$  which was larger than that of the intact product by Jet Mill pulverization (1.36

μm).

Besides, when the suspension containing nanoparticles (630 nm) (PHOTO C-1) was re-powdered by lyophilization, there was obtained a powder having a far larger mean particle size of 41.6 μm (the view of the product is shown in the attached Fig. 1, PHOTO C-2).

Thus, when a suspension containing AS-3201 particles was re-powdered by lyophilization, the smaller the mean particle size of the AS-3201 particle in the suspension, the larger the mean particle size of the lyophilized product. This shows that the smaller the mean particle size, the larger the flocculate force of the particles.

#### Experiment 5: Preparation of tablets

The following two kinds of tablets, Tablet A and Tablet B were prepared in the same formulation.

##### (1) Tablet A: Preparation of tablets by spray drying of suspension containing nanoparticles

As is disclosed in Experiments 3 and 4, a suspension containing nanoparticles was prepared by wet pulverization method, but it was impossible to take out the nanoparticles therefrom.

Accordingly, tablets containing AS-3201 which is assumed to be nanometer particles were prepared by spray drying of suspension containing AS-3201 particles prepared in Experiment 3(1) (20-Cycle, 630 nm) according to the following formulation (Table 4) and method.

**Table 4: Formulation of Tablet A**

Formulation	Amount	Content per each tablet
AS-3201	Particle-containing suspension prepared in Experiment 3(1) (corresponding to 40 g of AS-3201)	5 mg
Lactose	712g	89 mg
L-HPC	200g	25 mg
HPC	20g*	2.5mg
Tartaric acid	8g	1 mg
(Purified water)	(398g)	—
Mg stearate	20g	2.5 mg
Totally	1,000g	125 mg

\*: The amount of HPC was totally 20 g (including the amount (2 g) of HPC in the suspension)

**Method for preparing the tablets**

Hydroxypropylcellulose (HPC) and tartaric acid were dissolved in the suspension containing nanoparticles obtained in Experiment 3(1).

The resulting suspension was sprayed onto a mixture of lactose and low substituted hydroxypropylcellulose (L-HPC) in a fluid bed granulator and drier. The granules were dried, and thereto was added magnesium stearate, and the mixture was blended in a polyethylene bag. The resultant was compressed on a rotary tableting machine to give tablets.

**(2) Tablet B: Preparation of tablets by spray drying of suspension containing microparticles**

As a control tablet, Tablet B was prepared in the same manner as described in the above Experiment 5 (1) by spray drying of a suspension of microparticles, that is, a suspension prepared in Experiment 3(1) before pulverizing with DeBEE homogenizer (0-Cycle, 1.36  $\mu\text{m}$ ) instead of the suspension containing nanoparticles.

**Experiment 6: Study of dissolution properties of tablets**

The dissolution of the AS-3201 from Tablet A and Tablet B obtained in



Experiment 5 (1) and (2) was evaluated under the same conditions disclosed in the description of Serial No. 09/529,715. Each four tablets of A and B were tested according to Paddle method (50 rpm) specified in the Fourteenth Edition of the Pharmacopoeia of Japan, using a 0.2 M phosphate buffer (pH 6.5, 900 ml) as a test solution. The quantitative assay of AS-3201 was carried out by spectrophotometry at 300 nm. The results are shown in Table 5 and in the attached Fig. 2.

**Table 5** Dissolution rate of Tablet A and Tablet B

	Dissolution rate (%)			
	After 5 min.	After 10 min.	After 15 min.	After 30 min.
Tablet A : Tablets prepared by spray drying of suspension containing nano-particles (0.63 $\mu\text{m}$ )	79.7	93.8	97.3	99.5
Tablet B : Tablet prepared by spray drying of suspension containing micro-particles (1.36 $\mu\text{m}$ )	74.0	90.2	95.3	99.5

As is seen from Table 5 and Fig. 2, both of Tablet A and Tablet B showed substantially the same dissolution rate. Thus, there was no difference between Tablet A and Tablet B.

## 5. Conclusion

(1) I tried to prepare nanometer size particles by dry pulverization method, but failed (cf. Experiment 1).

(2) I tried to suspend microparticles of AS-3201 in water in order to prepare nanoparticles by wet pulverization method, but AS-3201 microparticles could not be dispersed without additives (cf. Experiment 2).

(3) Based on the finding that AS-3201 microparticles can be dispersed in water under the presence of HPC, a suspension containing AS-3201 microparticles and HPC (0-Cycle, 1.36  $\mu\text{m}$ ) (hereinafter, referred to as "microsuspension") was prepared and then subjected to wet pulverization to give a suspension containing nanoparticles (hereinafter, referred to as "nanosuspension") (20-Cycle, 630 nm) (cf. Experiment 3).

(4) I tried to take out powdery AS-3201 from a suspension containing

AS-3201 particles and got the following new findings.

(i) When the nanosuspension (20-Cycle, 630 nm) of the above (3) was subjected to vacuum drying, there were obtained particles having mean particle size of 102  $\mu\text{m}$ . Thus, it was failed to take out the desired nanoparticles by vacuum drying (cf. Experiment 4(1) ).

(ii) When the nanosuspension (20-Cycle, 630 nm) of the above (3) was subjected to freeze-drying, a powdery product could be obtained, but the powdery product had mean particle size of 41.6  $\mu\text{m}$ . Thus, it was failed to prepare the desired nanoparticles (cf. Experiment 4(2)(i) ).

(iii) When the suspension before pulverization as in the above (3), i.e. the microsuspension (0-Cycle, 1.36  $\mu\text{m}$ ) was freeze-dried, there were obtained particles having mean particle size of 8.2  $\mu\text{m}$  (Experiment 4(2)(ii) ).

(iv) From the above results in (ii) and (iii), it was proved that the smaller the mean particle size of AS-3201, the larger the flocculate force of the particles.

(5) Using two kinds of suspension prepared in the above (3), the following two kinds of tablets were prepared, and the dissolution rate of the tablets was measured. As a result, both tablets showed substantially the same dissolution rate (cf. Experiments 5 and 6).

Tablet A: Tablet prepared by spray drying of suspension containing nanoparticles (20-Cycle, 630 nm)

Tablet B: Tablet prepared by spray drying of suspension containing microparticles (0-Cycle, 1.36  $\mu\text{m}$ )

From the above experimental results, it will be clear that AS-3201 particles do not match to Noyes-Whitney Law.

I declare further that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 19 day of May, 2005

*Mamoru Ohashi*

---

Mamoru OHASHI

Fig. 1



PHOTO A

Jet Milled powder (1.36 $\mu$ m)

$\times 150$   
100 $\mu$ m

suspended in 0.5% HPC solution



PHOTO B-1

Suspension containing  
1.36  $\mu$ m-particles (0-cycle)

lyophilized

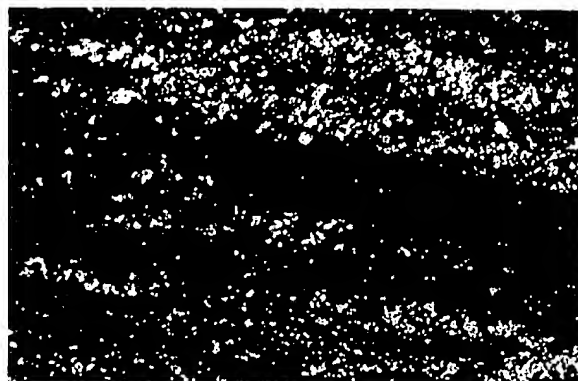


PHOTO B-2

Freeze dried powder (8.2 $\mu$ m)

grinded by DeBEE homogenizer

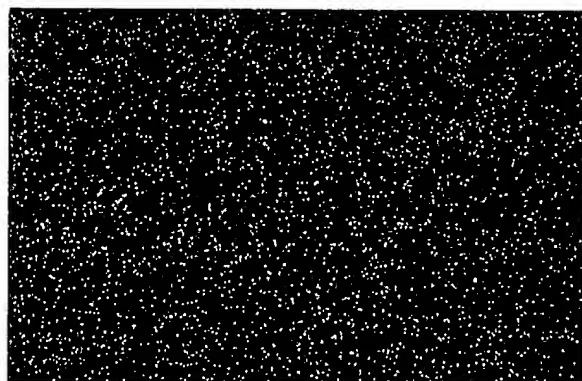


PHOTO C-1

Suspension containing  
0.63  $\mu$ m-particles (20-cycle)

lyophilized



PHOTO C-2

Freeze dried powder (41.6 $\mu$ m)

**Fig. 2**

